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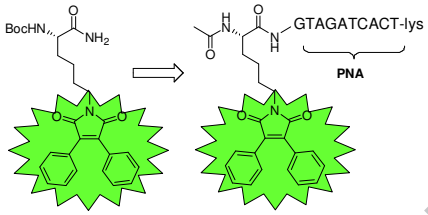

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Synthesis of luminescent 2,3-diphenylmaleimide-labelled peptide nucleic acid oligomers

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ABSTRACT

A lys-GTAGATCACT-lys peptide nucleic acid (PNA) decamer labelled with the luminescent 2,3-diphenyl maleimido (DPM) group on the ε-position of the terminal lysine residue was prepared through an automated solid phase synthesis. Fluorescence emission of the DPM-labelled PNA thus obtained was found to be significant and promising for the potential application in DNA recognition.

1. Introduction

Peptide nucleic acid (PNA) oligomers (Fig. 1), reported by Nielsen and co-workers in 1991,[1] are synthetic DNA mimics in which the negatively charged sugar–phosphate backbone is replaced by a neutral polyamide backbone composed of *N*-(2-aminoethyl) glycine units bearing the nucleobases. The PNAs have attracted significant attention due to their favourable properties such as increased stability in physiological media, high resistance to cellular enzymes, sequence-selective binding to complementary DNA/RNA[2] and excellent mismatch sensitivity.

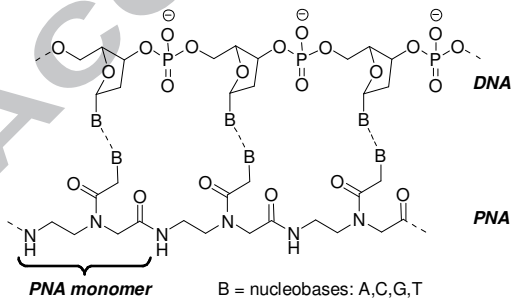

Figure 1. Schematic representation of a PNA polymer and its hybridization with complementary DNA

These features make PNAs exceptionally attractive for DNA recognition, and a large amount of research has been devoted to the use of PNAs either to detect gene expression or to be applied as antigene and antisense probes in different genosensors.[3-5] For applications as diagnostic probes, PNAs have to be equipped with reporter groups and a wide range of electrochemical,[6-7] fluorescent,[8] infrared[9] and radioactive groups[10] have been attached to PNA oligomers. Although many markers have been introduced on PNAs, the search for more efficient ones still continues. Following our previous research on the labelling of PNAs with different probes,[6,9] we turned our attention to the 2,3-diarylmaleimido group. Diarylmaleimido derivatives[11–13] exhibit a strong emission in the UV-visible range, but unlike other members of the carboxylic imide family,[14] namely phthalimide, naphthalimide and peryleneimide, have received less attention as markers for biomolecules. Various research groups have investigated the use of 2,3-diarylmaleimido derivatives for various applications, such as *n*-type organic semiconductors for thin film transistors and polymeric light-emitting diodes.[15-19] Herein, we describe the use of the 2,3-diphenyl maleimido (DPM) group as a new luminescent marker for PNA decamers as well as fluorescence analysis of one of the conjugates, which represents a potentially useful probe for DNA sensing.

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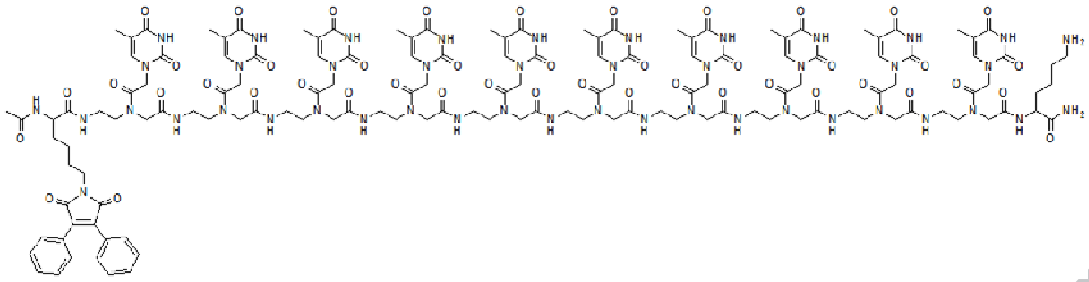


Figure 2. DPM-labelled-(LysT_{10}Lys)-PNA oligomer **5**

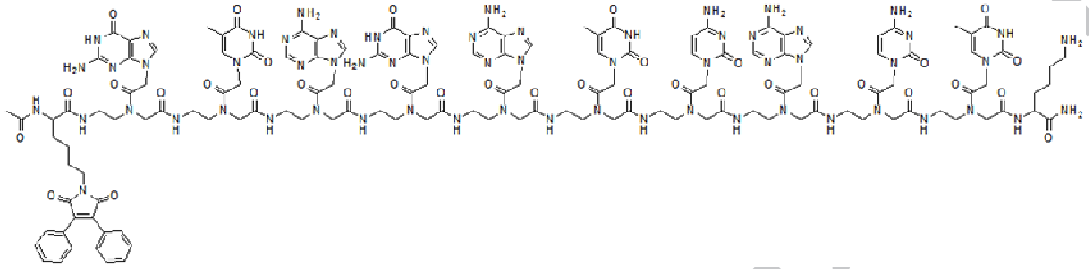
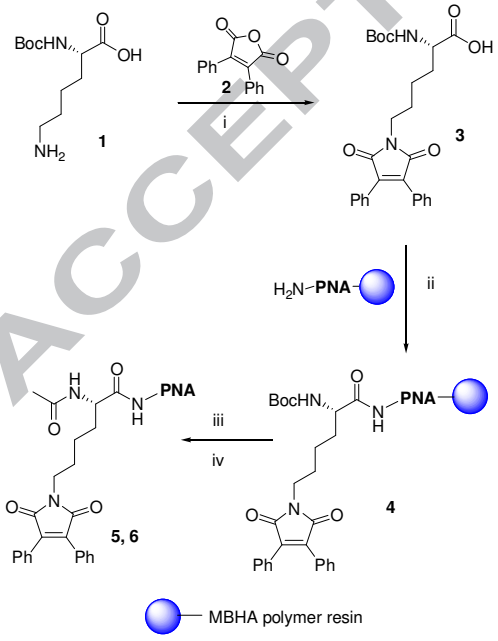


Figure 3. DPM-labelled (LysGTAGATCACTLys) PNA decamer **6**

2. Results and Discussion

The DPM-labelled PNA oligomers **5** and **6** (Fig. 2, 3) were synthesised as depicted in Scheme 1, namely by first introducing the DPM moiety to the ε-position of a lysine unit, thus giving **3**, which was subsequently linked to the *N*-terminus of a solid phase–supported PNA decamer. The target DPM-labelled PNA decamers were obtained after cleavage from the resin. In detail, the reaction between Nα-Boc-L-lysine **1** and 2,3-diphenyl maleic anhydride **2** was performed in the presence of catalytic 4-dimethylaminopyridine (DMAP) by heating at reflux in toluene; this process resulted in the formation of Nα-Boc-Nε-2,3-diphenyl maleimido-L-lysine **3** in 81% yield (Scheme 1).[20]



Scheme 1. i) TEA, DMAP, reflux, 15 h, 81%; ii) HBTU, NMP; iii) TFA then Ac_2O/Py; iv) TFA:TFMSA:m-cresol:thioanisole (6:2:1:1).

The UV absorption and emission of **3** were recorded in EtOH at different concentrations (Fig. 4). The absorbance maximum was observed at 365 nm and the emission maximum at 520 nm; **3** was detectable at very low concentrations (1 x 10^{-6} M).

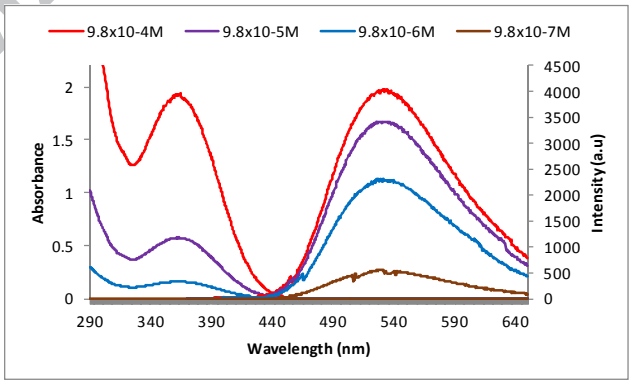


Figure 4. Absorption (λ_{max}= 365 nm) and Emission spectra (λ_{max}= 520 nm) of **3.**

The compatibility of DPM-lysine **3** with solid phase synthesis and the cleavage conditions was then verified by conjugating it to a homo-thymine–PNA decamer (Scheme 1). Since such a PNA oligomer does not possess biological significance, this typically represents an inexpensive and simple method to explore new reactions and reagents in PNA solid phase synthesis as well as cleavage conditions. The homo-thymine–PNA decamer was synthesized using standard solid phase technique on a MBHA polymer resin[21] loaded with an additional lysine at the C-terminus (see ESI). The labeling of the decamer was performed at the end of the sequence on solid phase by condensing DPM-lysine **3** in the presence of HBTU and NMP as solvent. After deprotection and acetylation of the α-nitrogen of the terminal DPM-lysine residue, the PNA decamer was cleaved from the resin using a mixture of TFA/TFMSA/m-cresol/thioanisole. The crude PNA oligomer **5** (Fig. 2) was purified using HPLC equipped with a semi-preparative column and the isolated oligomer was >95% pure. The MALDI-TOF analysis confirmed the presence of the DPM group that, subsequently, proved to be stable under cleavage conditions.

A similar protocol was followed to prepare a randomly chosen PNA oligomer **6** composed of different nucleobases (Fig. 3), which is more significant (representative) in view of further DNA recognition experiments. PNA **6** was isolated in 97% purity after purification by HPLC using a semi-preparative column. Similar to the earlier case, the MALDI-TOF analysis confirmed the presence of the labeling group on the PNA oligomer (see ESI).

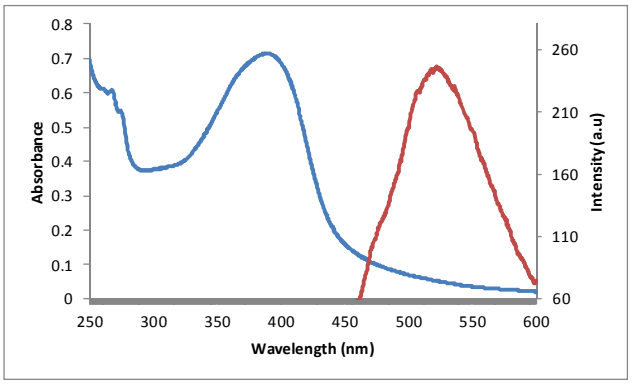


Figure 5. Absorbance (λ_{max} = 390 nm) and emission (λ_{max} = 520 nm) of DPM-PNA oligomer **6** , (9.8 x 10^{-6} M solution in EtOH).

In the view of using DPM-labelled PNA oligomers as active DNA probes, it is essential to evaluate their spectroscopic properties. Hence, UV absorption and emission measurements of labelled PNA decamer **6** were performed. The PNA **6** showed an absorbance maximum at 390 nm (Fig. 5) in UV light and a strong emission at 520 nm in ethanol solution (9.8 x 10^{-6} M). Further, the labelled PNA oligomer was detectable even at low concentrations (9.8 x 10^{-7} M). The quantum yield was calculated as per the standard protocols and was found to be 10.97%.

3. Conclusion

Starting from ε-diphenylmaleimido labelled lysine **3**, which is easy to prepare in high yield, new luminescent DPM-labelled PNA oligomers **5** and **6** were synthesized with high purity and favourable fluorescent properties. The attachment of the new fluorescent label **3** to PNA allows reasonable detection even at low concentration in UV. These preliminary results suggest that the diphenylmaleimido group can be considered as a promising candidate to tag PNA. Further experiments regarding DNA binding/recognition ability and cell uptake of DPM-labelled PNA are currently underway.

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20. Diphenyl maleic anhydride **2** (0.8 mmol), TEA (3.4 mmol) and a small amount of DMAP were added to a solution of *N*-Boc-L-lysine **1** (2.4 mmol) in toluene (10 mL). The reaction mixture was then heated at reflux for 15 h using a Dean and Stark apparatus. After distilling toluene, the residue was dissolved in ethyl acetate (50 mL), washed with $NaHCO_3$ (10 mL), then $KHSO_4$ (0.3 M solution, 10 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated to obtain the crude product, which was purified using a chromatographic column on silica gel (CH_2Cl_2) to give 81% of **3** as a pale green solid. ^{1}H NMR ($CDCl_3$, δ): 1.35 (s, 9H, CH_3-Boc); 1.59–1.68 (m, 6H, CH_2 Lys); 3.50–3.53 (m, 2H, CH_2-*N*-maleimide); 3.75 (1H, CH*); 7.32–7.35 (m, 10H, Ph). ^{13}C NMR ($CDCl_3$): 21.0, 28.2, 39.6, 77.7, 128.4, 128.6, 128.7, 135.9, 170.3. MS (ESI): m/z: 501.1987 (M+Na), 479.2137 (M+H). m.p: 86-90 °C
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Highlights

a. Synthesis of diphenylmaleimido-labelled lysine
b. Synthesis of diphenylmaleimido-labelled PNA oligomer
c. Promising luminescence behavior for labeled PNA oligomer